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The effect of addition of linoleic acid on food intake regulation in linoleic acid tasters and linoleic acid non-tasters

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In a randomised, single blind, placebo-controlled crossover design study, we investigated whether healthy, non-smoking, dietary unrestrained women (*n* 24), divided into linoleic acid tasters (LAT, *n* 14) and linoleic acid non-tasters (LANT, *n* 10), differed in food intake regulation when linoleic acid was added to ice creams. The determination of subjects as LAT or LANT was done using a 10 μM-linoleic acid solution. The ice creams were characterised by the subjects and a taste perception test using the triangle test was conducted three times. Food intake and appetite were measured using the universal eating monitor. LAT and LANT did not differ in characterisation or in taste perception of the ice creams, even though LAT were able to increase their ability to discriminate between the ice cream with linoleic acid from the one containing oleic acid. No effect of LAT status or type of ice cream was found for hedonic value of the ice creams. Linoleic acid taster status did affect food intake regulation. For LAT, but not LANT, the amount eaten was a function of Δsatiety. Subjects ate by weight of food and not by energy content. In conclusion, differences in food intake regulation were seen between LAT and LANT, in that the amount eaten by LAT was a function of Δsatiety, but was not for LANT.

Linoleic acid: Fat perception: Food intake regulation: Satiety: Macronutrients: Energy density

Many studies have examined the effect of fat intake in comparison with other macronutrients on satiety and/or food intake, without discriminating between different types of fat. Only a few studies investigated the effects of saturation of fats on satiety and found that fats containing linoleic acid were more satiating than fats containing oleic acid on equi-energetic basis (Lawton *et al.* 1997; French *et al.* 1998, 1999). In a recent study, we showed evidence for fat-specific satiety in that after a 2-week use of oil high in linoleic acid relative fat intake was lower at a dinner test meal with linoleic acid compared with a test meal with oleic acid after a 2-week use of oil high in oleic acid (Kamphuis *et al.* 2001). This evidence for fat-specific satiety appeared not to be related to taste perception of the different oils, but in fact we hypothesised that fat-specific satiety may be related to taste perception of different (non-esterified) fatty acids. This hypothesis is based upon studies by Gilbertson *et al.* (1998*b*). They found a relationship between perception of fatty acids in taste receptor cells and dietary preference for fat in rats. Gilbertson *et al.* (1997) demonstrated that non-esterified polyunsaturated fatty acids inhibit delayed rectifying K^+ channels in mammalian taste receptor cells, which in turn leads to an increase in activity of the taste cells. Interestingly, the effects were only seen for *cis*-polyunsaturated fatty acids (arachidonic, linoleic and linolenic acid), and not for the monounsaturated fatty acid oleic acid. Moreover, delayed rectifying K^+ channels in tongue tissue of Osborne–Mendel rats with a preference for high-fat diets were less sensitive to the *cis*-polyunsaturated fatty acids than delayed rectifying K^+ channels of S5B rats. The latter rats prefer diets high in carbohydrate (Gilbertson, 1998). Thus, an inverse relationship between fatty acid perception, i.e. linoleic acid perception and fat preference is seen in rats. Some evidence for a possible role of fat perception in human subjects has been shown by Tepper & Nurse (1998) and Tepper (1999). They found a relationship between 6-n-propyl thiouracyl (PROP) taster status and the ability to discriminate between differences in fat concentrations. Moreover, Nasser *et al.* (1999) investigated the role of PROP taster status on sensitivity to fat intake. They hypothesised that (PROP) tasters would be more sensitive to the presence of fatty acids and showed some evidence for this hypothesis in experiments in which linoleic acid was administered as part of conjugated linoleic acid added to high-fat ice cream. However, Tepper & Nurse (1998) and Tepper (1999) as well as Nasser *et al.* (1999) did not test whether subjects differed with respect to linoleic acid taste perception, and whether this was related to food intake regulation. In a previous study, we investigated whether subjects (*n* 221) were able to taste a low concentration of linoleic acid (10 μM). It was shown that 46 % of

Abbreviations: LANT, linoleic acid non-taster; LAT, linoleic acid taster; PROP, 6-*n*-propyl thiouracyl; VAS, visual analogue scale.

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the subjects were classified as linoleic acid tasters (LAT). Moreover, we showed that linoleic acid taste sensitivity might play a role in the aetiology of obesity (MMJW Kamphuis, 2003 unpublished results).

In the present study we tested whether there is a relationship between linoleic acid taste perception and food intake regulation in terms of food or energy intake, or satiety. Thus, we first distinguished LAT from linoleic acid non-tasters (LANT). Naturally, non-esterified linoleic acid is present in foods of which we are not aware, because we do not recognise it consciously. This phenomenon is similar to monosodium glutamate recognition. Despite lack of recognition, ingestion of non-esterified linoleic acid in foods may have consequences for food intake regulation. We therefore added linoleic acid in low concentrations to a food in order to study whether LAT and LANT would show a different response with respect to food intake regulation. We tested low- and high-energy foods containing linoleic acid, oleic acid or no addition of fatty acids.

Table 1. Baseline characteristics of the female subjects (Mean values and standard deviations)

	LAT (*n* 14)		LANT (*n* 10)	
	Mean	SD	Mean	SD
No. of correct answers†	10·0	0·0	6·8****	1·7
Age (years)	25·9	7·3	27·8	4·7
Weight (kg)	70·5	7·8	70·4	9·8
BMI (kg/m^2)	24·0	3·8	24·3	2·6
HP‡	10·9	3·2	13·1	2·3
F1 (restrained eating)§	3·8	2·9	5·6	4·2
F2 (disinhibition)§	3·8	2·8	4·4	3·4
F3 (hunger)§	3·6	2·8	5·0	3·4

LAT, lactic acid taster; LANT, lactic acid non-taster.
Mean value was significantly different from that of the LAT group: ****$P<0·0001$.
† Answers to the question: 'Which sample contains the fatty acid' to identify linoleic acid taster status. Subjects were characterised as LAT when given nine or more correct answers out of ten or as LANT when given less than nine correct answers.
‡ Herman–Polivy restraint score (for details, see Herman & Polivy (1980)).
§ Three factor eating questionnaire score (for details, see Stunkard & Messick (1985) and Westerterp-Plantenga *et al.* (1999)).

Material and methods

Subjects

Thirty-five women were recruited by advertisements in local newspapers. Twenty-four subjects were selected for the experiment. Selection was based upon being healthy and at least 3 months weight-stable prior to the study, not using any medication known to affect body weight and/or appetite, being non-smoking, and at most moderate alcohol-users (maximum ten glasses per week). Subjects had to be unrestrained eaters. The degree of dietary restraint was determined by the three factor eating questionnaire (score F1 $\leq$ 9) (Stunkard & Messick, 1985; Westerterp-Plantenga *et al.* 1999) and by the Herman–Polivy restraint questionnaire (score $\leq$ 15) (Herman & Polivy, 1980). Body weight (after an overnight fast) was measured on a digital balance (model 707; SECA, Hamburg, Germany, weighing accuracy 0·01 kg) while subjects were wearing underwear. Body weight was measured before the study had started. Height was measured to the nearest 0·001 m using a wall-mounted stadiometer (SECA) and BMI (kg/m^2) was calculated as body weight (kg) divided by height (m) squared. Furthermore, all subjects completed a linoleic acid taster test to divide them into LAT and LANT (see below for linoleic acid taste perception test; Kamphuis *et al.* 2003). Table 1 shows the baseline characteristics of the twenty-four subjects divided in LAT and LANT. All subjects gave their written informed consent. The study was approved by the Medical Ethics Committee of Maastricht University.

Linoleic acid taste perception test

Linoleic acid taster status was determined as follows. Linoleic acid (sodium salt) was diluted with propylene glycol and de-mineralised water (10 μM), while the placebo solution contained only propylene glycol and de-mineralised water. The concentration of 10 μM is identical to the concentrations of non-esterified fatty acids that Gilbertson *et al.* (1997, 1998*b*) used in the rat studies and as we used in a previous study (MMJW Kamphuis, unpublished results). Twelve pairs of two samples were offered with one sample containing 10 μM-linoleic acid and the other containing the solution. The first two pairs were practice samples and were not included in the score. Subjects were asked to taste and expectorate half of the first sample, rinse with water, taste and expectorate half of the second sample, rinse with water, taste and expectorate the remaining first sample, rinse with water and to taste and expectorate the remaining of the second sample. After tasting each sample twice, i.e. after tasting a pair of two samples, subjects had to answer the question: 'Which sample contains fatty acids?'. Between pairs, subjects had to rinse with water twice. With the linoleic acid taste perception test, a distinction between 10 μM LAT and LANT could be made as follows. If subjects were not able to distinguish the fatty acid from the placebo solution, and so had to guess (P=0·5, n 10), the chance of guessing ten correct answers is $<$0·1 %. The chance of guessing more than nine correct answers out of ten is 1·1 %, while the chance exceeds the 5 % level with guessing eight or more correct answers out of ten pairs. Thus, subjects were characterised as LAT when they gave nine or more correct answers out of ten and as LANT when they gave less than nine correct answers. Subjects were tested twice, with 1 week in between the tests. The outcome of the second test was used in order to identify subjects as LAT and LANT. In a previous study, we showed that the reproducibility of the linoleic acid perception test was 95 % (Kamphuis *et al.* 2003).

PROP taster status

PROP taster status of the subjects was identified as follows. Five concentrations of NaCl (0·01–1·00 M) increasing in half-log steps in random order and five concentrations of PROP ($3·2\times10^{-5}$–$3·2\times10^{-3}$ M) increasing in half-log steps in random order were rated using a 150 mm visual analogue scale (VAS). Samples were tasted and expectorated.

Subjects rinsed with water in between each sample until the taste of PROP or NaCl was disappeared. This procedure was used to generate suprathreshold taste intensity functions for the two compounds. When these two functions were superimposed and the slope of the PROP curve appeared much lower than the slope for the NaCl curve, a subject was classified as a PROP non-taster. When the PROP curve overlapped with the NaCl curve, a subject was classified as a PROP medium taster. A subject was classified as a PROP supertaster when the slope of the PROP curve was steeper than the NaCl slope (Bartoshuk 1994; Kamphuis *et al.* 2001).

Experimental design

The study was a single blind, randomised placebo-controlled trial, which consisted of six test days. The subjects came six times on the same day of the week and the same time of the day to our department after a 3 h fast, for a test. During each test, one type of ice cream was offered *ad libitum* from the universal eating monitor (Westerterp-Plantenga, 2000) in random order. Before this *ad libitum* test meal, subjects were asked to characterise the type of ice cream they were offered on that day using a 25 g sample (see p. 201 for test protocol for characterisation of the ice creams). To assess whether energy content or type of ice cream with respect to fatty acid content would influence energy intake or satiety, variables of meal consumption and appetite for six different ice creams were determined during each test (see p. 202 for test protocol eating profile). Moreover, to assess whether subjects were able to distinguish between ice creams, a taste perception test for the ice creams was conducted during the last four visits (see p. 203 for test protocol taste perception).

Non-esterified linoleic acid is present in foods in low concentrations, although we do not taste it and we are not aware of its presence. In the present study, non-esterified linoleic acid was added in low concentrations to study whether LAT and LANT would show a different response with respect to food intake regulation. Linoleic acid was offered as a non-esterified fatty acid, which is present in conjugated linoleic acid at a concentration of 3 g/100 g total fatty acids. Since non-esterified fatty acids oxidise rapidly at room temperature, the study was conducted with ice creams in order to minimise oxidation. The low-energy ice cream with linoleic acid was tested against a low-energy ice cream without linoleic acid and a low-energy ice cream with oleic acid. Moreover, the low-energy ice cream with linoleic acid was tested against a high-energy ice cream with linoleic acid. The latter was tested against a high-energy ice cream without linoleic acid and a high-energy ice cream with oleic acid.

The non-esterified fatty acid and triacylglycerol composition of the ice creams were analysed with the following procedure. Lipids were extracted according to the method of Folch *et al.* (1957). TLC plates were used to separate non-esterified fatty acids and triacylglycerols from the total lipid extract (Kaluzny *et al.* 1985). The fatty acids were transmethylated to the corresponding methyl esters by reaction with acetylchloride and methanol at 100°C for 1 h (Lepage & Roy, 1986). The fatty acid methyl esters were separated and quantified by using a HP 5890 II G-C, fitted with a 50 m CP sil88 capillary column with 0·25 mm internal diameter and 0·12 μm film thickness (Chrompack®, Middelburg, The Netherlands). A standard fatty acid methyl ester mixture was used to identify the fatty acid methyl esters by means of the retention times. The non-esterified fatty acid and triacylglycerol composition of the ice creams are presented in Table 2.

Ice cream

One unit for the low-energy ice creams contained 1 litre low-fat milk, 250 g sugar, 5 g coffee powder (Nescafe Mild, Nestle, Lausanne, Switzerland), 15 g cocoa (Blooker, Amsterdam, The Netherlands) and 3 g thickener (Mobexgel F500; Black B.V., De Meern, The Netherlands). In the high-energy ice creams, the low-fat milk was replaced by 500 ml full-fat milk and 500 ml cream.

The milk products (low-fat milk or high-fat milk with cream) together with the sugar, coffee powder, cocoa and thickener were heated until the boiling point. After that, they were cooled and stored at 4°C for 24 h before making the ice creams. Just before making ice cream, linoleic acid (0·03 g linoleic acid per unit), placebo (oleic acid) or nothing was added.

The low-energy ice creams contained 4·6 kJ/g and had a macronutrient composition of 11 % energy as protein, 2 % as fat and 87 % as carbohydrate. The high-energy ice creams contained 10·1 kJ/g and had a macronutrient composition of 4 % energy as protein, 58 % as fat and 38 % as carbohydrate. Addition of linoleic acid or oleic did not change macronutrient composition.

The ice creams were always produced by the same experimenter in the week preceding the test days. The ice creams were stored at −20°C in 750 ml dishes. One hour before tests, the ice cream was taken out the freezer and put in a fridge, so that during the test meal the ice creams had an acceptable temperature (± −2°C) and texture.

Test protocol for characterisation of the ice creams

Before the subjects started a test meal, they tasted and characterised 25 g ice cream they were offered that day (Kamphuis *et al.* 2001). They were asked: 'How sweet, bitter, sour, salty, neutral, full of taste and how creamy is

Table 2. Linoleic acid and oleic acid content (μg/mg ice cream) of the ice creams as non-esterified fatty acid (NEFA) and as triacylglycerol (TG)*

	L	LL	LO	H	HL	HO
NEFA linoleic acid	0·00	0·01	0·00	0·01	0·03	0·01
NEFA oleic acid	0·01	0·1	0·01	0·12	0·17	0·31
TG linoleic acid	0·1	0·2	0·1	1·14	2·5	2·2
TG oleic acid	1·0	1·0	1·8	16·6	25·3	29·7

L, low-energy ice cream without linoleic acid; LL, low-energy ice cream with linoleic acid; LO, low-energy ice cream with oleic acid; H, high-energy ice cream without linoleic acid; HL, high-energy ice cream with linoleic acid; HO, high-energy ice cream with oleic acid.

* For details of analytical procedures, see p. 200.

the ice cream?' and 'How much do you like the ice cream?'. The subjects scored every question on a 100 mm VAS anchored 'not at all' on the left and 'extremely' on the right.

Test protocol eating profile

During the six times the subjects came to the department, they ate the different ice creams in random order (one type each test) from the universal eating monitor. Meal size, meal duration and eating rate were measured, as well as satiation during the meal.

Before and every 90 s during the test meal subjects were asked: 'How satiated are you?' and 'How strong is your desire to eat this ice cream?' Fifteen seconds after starting the meal and after termination of the meal, they were asked how pleasant the taste of the ice cream was at that moment. The subjects rated the answers on these questions on a VAS, which appeared on a computer screen in front of the subjects. They were instructed to answer the questions in between bites, so not to disrupt their eating rate.

Test protocol taste perception

The ability of subjects to discriminate between the ice cream with linoleic acid against no addition, and with linoleic acid against oleic acid was tested using the triangle test. This test is effective for determining if overall differences exist. Moreover, it can select subjects for ability to discriminate differences (Meilgaard *et al.* 1991).

In each trial three samples of ice cream were offered: two the same and one different. Subjects had to taste each sample and expectorate it. Between each sample subjects rinsed their mouth with bread and water, which had to be expectorated also. The procedure of a trial of three samples had to be repeated before the subjects answered the question: 'Which ice cream is the odd one out?'.

Four tests were conducted over 4 weeks. During the first test, subjects had to test ice cream with linoleic acid against ice cream without linoleic acid in order to test if the subjects were able to distinguish low-energy ice cream with linoleic acid from low-energy ice cream without linoleic acid. During the last three tests, ice cream with linoleic acid was tested against ice cream with oleic acid. After the second test, i.e. the first one testing low-energy ice cream with linoleic acid against low-energy ice cream with oleic acid subjects took both ice creams home. They were asked to practice the test at home every day. With this arrangement we were able to test the learning effect of the discrimination.

Statistical analysis

The relationship between linoleic acid taster status and PROP taster status was analysed with a χ^2 test. For the test protocol taste perception, subjects had to correctly identify five samples out of six in order to say that they were able to discriminate the linoleic acid-containing ice cream from the ice cream with oleic acid or from the ice cream with no addition. Improvements in taste perception between the ice cream with linoleic acid and the ice cream with oleic acid for LAT and LANT were analysed with a two-factor repeated-measures ANOVA. *Post hoc* analysis was executed with a Scheffé *F* test (Statview SE Graphics™, Berkeley, CA, USA). Possible differences in characterisation, food and energy intake, meal duration, eating rate and appetite profile between LAT and LANT for the individual ice creams were analysed with a two-factor repeated-measures ANOVA. *Post hoc* analysis between ice creams was done with the Scheffé *F* test (Statview SE Graphics™) and between linoleic acid taster groups with an unpaired *t* test (Statview SE Graphics™). The relationship between amount eaten (g and kJ) and Δsatiety as well as between Δpleasantness of taste and hedonic value were tested with a simple regression (Statview SE Graphics™).

The data are presented as mean values and standard deviations. The level of significance was set at $P<0{\cdot}05$.

Results

Subject characteristics

Of the twenty-four female subjects, fourteen subjects were characterised as LAT and ten as LANT. These two groups in the present study did not differ in age, body weight, BMI and dietary restraint (Table 1).

Of the LAT group 63 % and of the LANT 60 % were PROP tasters. There was no relationship between linoleic acid taster status and PROP taster status (results not shown).

Characterisation of the ice creams

LAT and LANT did not differ in characterisation of the ice creams (Table 3). Moreover, no differences in characterisation with respect to sweet, sour, salty and neutral taste between the ice creams were seen (Table 4). However, differences between ice creams for 'bitterness', 'full of taste' and 'creaminess' were observed. Addition of linoleic acid made the high-energy ice cream less bitter and more full of taste and creamy compared with high-energy ice cream without addition of any fatty acid. Moreover,

Table 3. Characterisation of the six ice creams together (visual analogue score, mm) by female subjects divided in linoleic acid tasters (LAT) and linoleic acid non-tasters (LANT)*

(Mean values and standard deviations)

	LAT (*n* 14)		LANT (*n* 10)	
	Mean	SD	Mean	SD
Sweet	52·3	17·0	53·1	12·0
Sour	12·1	12·3	5·4	5·6
Salty	15·6	14·4	11·9	13·1
Bitter	29·0	18·1	16·3	11·5
Neutral of taste	32·7	14·2	39·1	17·8
Full of taste	56·0	10·0	55·1	11·7
Creamy	52·7	13·4	52·8	12·4
Hedonic	46·3	16·5	56·1	18·0

* For details of subjects, ice creams and procedures, see Tables 1 and 2 and pp. 200–201.

subjects found high-energy ice cream with linoleic acid more full of taste and more creamy compared with the low-energy variant. Furthermore, the high-energy ice cream with oleic acid was observed to be more creamy than the high-energy ice cream without addition of any fatty acid as well as the low-energy ice cream with oleic acid. The latter, in turn, was observed to be more creamy than the low-energy ice cream with linoleic acid (Table 4). No differences in hedonic value between ice creams, or between linoleic acid taster groups were seen. There was no taster × ice cream interaction for any variable.

Eating profile

No differences in meal size, meal duration or eating rate were seen between the types of ice creams or between LAT and LANT, nor was there a taster × ice cream interaction. Therefore, these variables of all types of ice creams are taken together (Table 5).

The amount eaten (g) did not differ between ice creams. Because of the differences in energy content, the amount eaten expressed as energy (kJ) differed between the ice creams. Subjects ate more from the high-energy ice creams than from the low-energy ice creams (high-energy without linoleic acid 1649·9 (SD 615·9), high-energy with linoleic acid 2069·0 (SD 944·9) and high-energy with oleic acid 2012·4 (SD 929·0) *v.* low-energy without linoleic acid 854·4 (SD 529·5), low-energy with linoleic acid 889·3 (SD 437·5) and low-energy with oleic acid 910·5 (SD 409·4) kJ, $P<0·0001$). Moreover, within the high-energy ice creams, subjects ate more from the high-energy ice cream with addition of linoleic acid compared with the high-energy ice cream without addition of a fatty acid ($P<0·0001$).

Because the satiety levels before and at the end and Δsatiety did not differ between ice creams or between LAT and LANT, the results are presented as mean values (Table 5). The difference with respect to food intake regulation between LAT and LANT consisted of an interaction between linoleic acid perception and Δsatiety ($F(3,54)$ 3·0, P=0·039). For the LAT there was a strong positive relationship between amount eaten (g and kJ) and Δsatiety for low-energy with linoleic acid (r^2 0·6, $P<0·001$; Fig. 1), high-energy without linoleic acid (r^2 0·6, $P<0·001$), high-energy with oleic acid (r^2 0·5, $P<0·01$), and a trend for high-energy with linoleic acid (r^2 0·2, $P<0·08$). In contrast, no relationship for any ice cream between amount eaten (g and kJ) and Δsatiety was seen for the LANT.

No differences between LAT and LANT were observed with respect to the pleasantness of taste levels before and after the meal. However, Δpleasantness of taste with the low-energy ice cream with addition of linoleic acid was higher for LANT (VAS −20·5 (SD 13·5) mm) compared with LAT (VAS −9·6 (SD 13·5) mm), but did not reach the level of significance ($P<0·1$). Between ice creams, the pleasantness of taste levels after the meal was lower with high-energy without linoleic acid (VAS 30·1 (SD 23·3) mm) compared with high-energy with linoleic acid (VAS 54·4 (SD 24·7) mm, $P<0·001$) and high-energy with oleic acid (VAS 55·2 (SD 29·2) mm, $P<0·001$). Moreover, the Δpleasantness of high-energy without linoleic acid (VAS −28·2 (SD 23·7) mm) was greater than the Δpleasantness of taste with high-energy with linoleic acid (VAS −11·3 (SD 15·4) mm $P<0·01$).

Taste perception

Because in general subjects gave less than five correct answers in each taste perception test, they were not able to discriminate consciously between the different ice creams. However, large individual differences were seen, but there was no relationship between the number of correct answers on the taste perception test (linoleic acid *v.* no addition and linoleic acid *v.* oleic acid) and number of correct answers on the linoleic acid perception test. Interestingly, LAT significantly increased their abilities to discriminate the ice cream with linoleic acid from the ice cream containing oleic acid from the first to the third test (2·7 (SD 1·3) *v.* 3·9 (SD 1·1), $P<0·05$), but did not differ from LANT. ANOVA showed significance for repeated

Table 4. Characterisation of the six ice creams (visual analogue scale, mm) for female linoleic acid tasters and linoleic acid non-tasters together‡

(Mean values and standard deviations)

	L		LL		LO		H		HL		HO		Statistical significance of effect: *P*
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Sweet	55·2	18·6	47·7	19·9	52·9	16·5	50·0	20·6	53·2	19·0	56·8	17·9	NS
Sour	11·5	16·8	9·3	11·6	9·5	9·7	10·5	14·1	5·9	9·0	10·3	16·3	NS
Salty	13·6	17·4	15·6	18·7	11·7	13·4	18·8	24·1	12·1	14·9	13·2	18·2	NS
Bitter	25·5	24·9	22·1	22·3	26·1	24·9	34·3	24·4	16·2*	17·8	20·0	20·9	<0·01
Neutral of taste	37·5	16·9	40·0	21·8	37·2	24·0	29·5	18·8	33·9	19·1	33·2	23·8	NS
Full of taste	51·1	19·7	40·5	18·7	53·1	16·0	52·5	18·7	68·9*†	15·3	67·5	19·5	<0·0001
Creamy	47·0	17·5	32·4	23·2	49·3†	15·7	46·3	19·9	69·7*†	17·1	71·6*	20·2	<0·0001
Hedonic	50·9	22·9	43·6	23·7	50·0	21·2	44·6	18·3	56·0	18·9	55·8	24·5	NS

L, low-energy ice cream without linoleic acid; LL, low-energy ice cream with linoleic acid; LO, low-energy ice cream with oleic acid; H, high-energy ice cream without linoleic acid; HL, high-energy ice cream with linoleic acid; HO, high-energy ice cream with oleic acid.

*Mean value was significantly different from those of the H group.

†Mean value was significantly different from those of the LL group.

‡For details of subjects, ice creams and procedures, see Tables 1 and 2 and pp. 200–201.

Table 5. Eating and appetite profile variables presented as the average of the six ice creams together by female subjects divided in linoleic acid tasters (LAT) and linoleic acids non-tasters (LANT)*

(Mean values and standard deviations)

	LAT (*n* 14)		LANT (*n* 10)	
	Mean	SD	Mean	SD
Meal duration (s)	500·6	156·4	399·9	182·7
Amount eaten (g)	200·5	81·2	175·5	81·9
Amount eaten (kJ)	1316·7	534·1	1140·2	534·5
Eating rate (g/s)	0·4	0·2	0·5	0·2
Bite size (g)	4·2	1·2	4·8	2·1
Bite frequency (bites per s)	0·1	0·0	0·1	0·0
Satiety (*t* 0)†	19·0	11·3	20·0	18·3
Satiety (end)†	84·7	8·3	82·7	9·1
ΔSatiety†	64·2	14·6	62·8	22·5
Pleasantness of taste (*t* 0)†	56·5	18·1	64·8	20·8
Pleasantness of taste (end)†	42·9	21·6	44·7	17·5
ΔPleasantness of taste†	−14·5	12·2	−20·1	10·6

* For details of subjects, ice creams and procedures, see Tables 1 and 2 and pp. 200–201.
† Visual analogue scale, mm.

measurements; LAT gave more correct answers with linoleic acid *v*. no supplementation than with linoleic acid *v*. oleic acid during its first test ($P<0·01$).

Discussion

In general, we observed in a group of 221 subjects that 46 % of the subjects could be classified as 10 μM LAT (Kamphuis *et al.* 2003). In the present study, we selected fourteen LAT and ten LANT to participate in the study. Subjects had to give nine or more correct answers out of ten, to be characterised as LAT. Next to the correct answer on the test pair, we also asked how sure subjects were about their answer. All subjects who were characterised as LAT said they were absolutely sure about their answers, while the LANT were not. So, even though LANT scored higher than a random guess (6·8 instead of 5·0), they were not able to taste it consciously, as the LAT did.

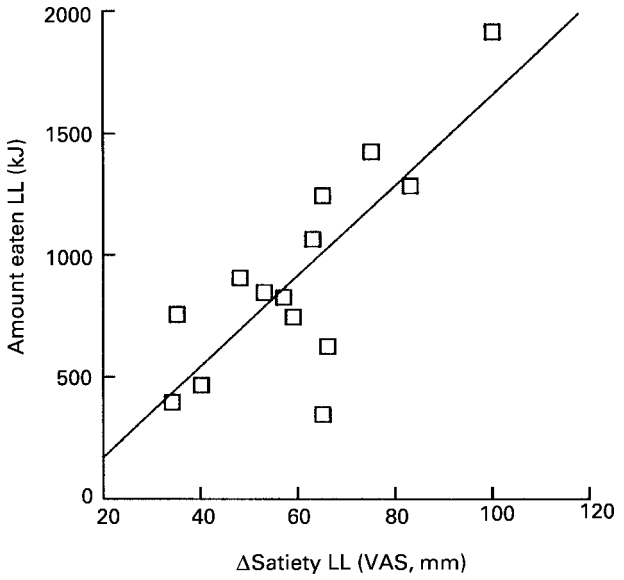

Fig. 1. Relationship between amount eaten of a low-energy ice cream with linoleic acid (LL) and Δsatiety levels for fourteen female subjects who were characterised as fatty acid tasters. VAS, visual analogue scale. r^2 0·6, $y=18·6\times-194·9$, $P<0·001$. For details of subjects, ice creams and procedures, see Tables 1 and 2 and pp. 200–201.

From the taste perception tests, it was shown that LAT increased their ability to discriminate between a low-energy ice cream with linoleic acid and a low-energy ice cream with oleic acid. This indicates a relatively higher sensitivity to a low concentration of linoleic acid in food of LAT compared with LANT. In LAT, in contrast to LANT, the amount eaten was a function of Δsatiety, particularly for the low-energy ice cream with non-esterified linoleic acid and the high-energy ice creams, which also contained non-esterified linoleic acid. In contrast, LANT showed a tendency for a greater Δpleasantness of taste compared with LAT from before to after eating the low-energy ice cream with addition of linoleic acid. This finding implies that linoleic acid perception may play a role in food intake regulation, in that it may explain different reasons for terminating a meal. In addition, the relationships between Δsatiety and the amount eaten only observed in the LAT imply a more general sensitivity for fat in tasters. This is also confirmed by the generally higher scores on characteristics of the ice creams by the tasters.

Since in LAT all high-energy ice creams which contained non-esterified linoleic acid were shown to affect the relationship between Δsatiety and amount eaten, as well as the low-energy ice cream with addition of linoleic acid, it can be suggested that addition of linoleic acid to low-energy foods might affect food intake regulation in LAT to the same extent as high-energy foods.

In our present study, only LAT terminated their meals consisting of ice creams because they were satiated. With this observation, we showed that linoleic acid taster status might affect food intake regulation. Although this was limited to showing the mechanism, i.e. amount eaten was a function of satiety and was not extended to the amount eaten itself, we suggest that it still gives evidence for supporting the Gilbertson hypothesis (Gilbertson, 1998). The satiating capacity might not only take place through tasting, since delayed rectifying K^+ channels also have been discovered in other parts of the gastrointestinal tract (Gilbertson *et al.* 1998*b*).

In the present study, LAT and LANT differed with respect to the relationship between amount eaten (kJ) and satiety. Hetherington (1996) investigated reasons for ending a course of a meal of a two-course test. After the first course, sensory-specific satiety (Rolls, 1986) was the most important reason for ending a meal, while after the second course, which was offered *ad libitum* 1 hour later, satiety and/or fullness determined termination of the meal. So, different reasons might be experienced by subjects for ending a meal. This also indicates that for satiety the signal needs to be stronger than for sensory-specific satiation. Therefore, when the amount eaten is a function of Δsatiety as in the LAT, termination of the meal might be more definite than in the LANT.

Food and energy intake did not differ between LAT and LANT, but differences between energy intake from low-energy and high-energy ice creams were observed. Subjects ate more from the high-energy ice creams than from the low-energy ice creams when expressed as energy intake (kJ), but not when expressed on a weight basis (g). This showed again that subjects regulated their food intake on the short-term through consumption by weight of food and not by energy content (Lissner *et al.* 1987; Blundell *et al.* 1995; Stubbs *et al.* 1995; Westerterp-Plantenga, 2000, 2001), which might easily lead to a passive over-consumption of energy from foods with a high energy density (Blundell *et al.* 1995; Blundell & MacDiarmid, 1997). Next to energy density of a food, palatability plays an important role in food intake (Drewnowski, 1998). Since the hedonic value did not differ between ice creams, the palatability was not a confounder in the present study.

In the present study, a small texture effect was shown. With respect to oral texture sensation, it appeared that high-energy ice creams were perceived as more creamy when fatty acids were added. Subsequently, subjects ate more from the high-energy ice cream with addition of linoleic, which was perceived as more creamy, than from the high-energy ice cream without addition of a fatty acid.

The concentration of the added linoleic acid to the ice creams was 1·4 mM while the linoleic acid taster test was conducted with solutions of 10 μM. Even though the concentration in the ice creams was 140-fold greater than in the solutions, the taste intensity may be masked, since the ice creams contained many compounds (e.g. cocoa, coffee) masking the taste of the fatty acid. This may explain why the ice creams were not clearly different perceived or characterised.

In an earlier study, we found evidence for fat-specific satiety for oils high in linoleic acid compared with oils high in oleic acid (Kamphuis *et al.* 2001). However, this fat-specific satiety was not related to taste perception of the different oils. Because we used oils for the taste perception test in that study, we tested whether subjects were able to perceive triacylglycerol instead of non-esterified fatty acids. Triacylglycerols need to be hydrolysed by lipases in order to become non-esterified fatty acids. The presence of lingual lipase in human subjects has been demonstrated (Hamosh *et al.* 1975; Hamosh & Burns, 1977) and activity has been detected (Spielman *et al.* 1993); however, the physiological role is uncertain. Possible activity of lingual lipase in human subjects remains to be demonstrated in relation to linoleic acid perception in oils. However, several foods contain non-esterified fatty acids, so breaking down triacylglycerol by lingual lipase is not the only prerequisite for a function of fatty acid perception in satiety.

In the present study we used non-esterified linoleic acid for the taste perception test and we found differences concerning food intake regulation between subjects who perceive the linoleic acid compared with subjects who do not. However, no relationship was seen between the number of correct answers on the linoleic acid taster test and on the taste perception test. This might be due to the fact that subjects, LAT as well as LANT were not able to consciously discriminate between ice creams with linoleic acid and ice creams with oleic acid or no fatty acid. The finding that LAT increased their ability to discriminate between the ice cream with linoleic acid from the one containing oleic acid and the fact they gave more correct answers in the taste perception test with linoleic acid *v.* no supplementation shows that LAT are more sensitive for the linoleic acid than LANT.

It has been hypothesized that sensitivity for fat and fat perception might be related to the ability to sense PROP. Tepper & Nurse (1997, 1998) and Tepper (1999) found that PROP tasters had an increased ability to discriminate between salad dressings with different fat content. Moreover, Nasser *et al.* (1999) found that PROP tasters were more sensitive to the presence of conjugated linoleic acid in ice cream than PROP non-tasters. However, they did not investigate whether PROP taster status was related to the ability to perceive non-esterified linoleic acid. In contrast to these findings, neither in the present study nor in our earlier study (*n* 221) (Kamphuis *et al.* 2003), PROP taster status was related to linoleic acid taster status. In support of our present conclusion, Yackinous & Guinard (2001) found that PROP taster status was not related to fat perception. Recently, the family of bitter receptors in the tongue has been discovered (Firestein, 2000), and appeared not to be related to the delayed rectifying K^+ channels involving the fat perception.

In conclusion, differences in mechanisms of food intake regulation were observed between LAT and LANT. In LAT, but not LANT, the amount eaten was a function of Δsatiety, indicating a relationship between taste perception of linoleic acid and a determinant of food intake regulation. Taken together, addition of linoleic acid to low-energy ice creams triggers a satiety mechanism in LAT. This effect is comparable with high-energy ice creams with non-esterified linoleic acid.

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